

Appl. No. 10/027,000
Amdt. dated May 5, 2004
Reply to Office action of February 5, 2004

Page 4

IN THE CLAIMS:

The claims as currently presented and under consideration, are presented below for the Examiner's convenience and to comply with 37 CFR §1.121:

1. [Cancelled]
2. [Currently Amended] An isolated polynucleotide from a fungal source, which polynucleotide comprises a nucleotide sequence encoding an enzyme having β -glucosidase IV ~~endo~~glucanase activity selected from the group consisting of:
 - (a) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a β -glucosidase 4 polypeptide having at least 85% sequence identity to the amino acid sequence presented in Figure 2-(SEQ ID NO:2);
 - (b) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a β -glucosidase 4 polypeptide having at least 90% sequence identity to the amino acid sequence presented in Figure 2-(SEQ ID NO:2);
 - (c) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a β -glucosidase 4 polypeptide having at least 95% sequence identity to the amino acid sequence presented in Figure 2;
 - (d) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a β -glucosidase 4 polypeptide having the amino acid sequence presented in Figure 2;
 - ~~(e) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a β -glucosidase 4 polypeptide having at least 95% sequence identity to the amino acid sequence presented as SEQ ID NO:2;~~
 - ~~(f) a nucleic acid sequence which encodes or is complementary to a sequence which encodes a β -glucosidase 4 polypeptide having the amino acid sequence presented as SEQ ID NO:2;~~
 - (ge) a nucleic acid sequence presented as SEQ ID NO:3, or the complement thereof; and
 - (hf) a nucleic acid sequence that hybridizes, under high stringency conditions to the sequence presented as SEQ ID NO:3, or the complement or a

Appl. No. 10/027,000
Amdt. dated May 5, 2004
Reply to Office action of February 5, 2004

Page 5

fragment thereof, wherein said isolated polynucleotide encodes a polypeptide having the biological activity of a β -glucosidase

wherein % identity is calculated using the CLUSTAL-W program in MacVector version 6.5, operated with default parameters, including an open gap penalty of 10.0, an extended gap penalty of 0.1, and a BLOSUM 30 similarity matrix.

3. [Cancelled]
4. [Original] The isolated polynucleotide of Claim 2, wherein hybridization is conducted at 42°C in 50% formamide, 6X SSC, 5X Denhardt's solution, 0.5% SDS and 100 μ g/ml denatured carrier DNA followed by washing two times in 2X SSPE and 0.5% SDS at room temperature and two additional times in 0.1 SSPE and 0.5% SDS at 42°C.
5. [Original] The isolated polynucleotide of Claim 2, wherein said polynucleotide is an RNA molecule.
6. [Currently Amended] The isolated polynucleotide of claim 2 encoding an enzyme having β -glucosidase activity, wherein the enzyme is isolated ~~derived~~ from a *Trichoderma* source.
7. [Currently Amended] The isolated polynucleotide of Claim 6, wherein the enzyme is isolated ~~derived~~ from *Trichoderma reesei*.
8. [Currently Amended] An expression construct comprising ~~including~~ a polynucleotide sequence (i) having at least 85% sequence identity to the amino acid sequence presented in Figure 2-(SEQ ID NO:2), or (ii) being capable of hybridizing to a probe designed to the nucleotide sequence encoding the amino acid sequence disclosed in Figure 2 under conditions of intermediate to high stringency, or (iii) being complementary to a nucleotide sequence encoding the amino acid sequence having at least 85% sequence identity to the amino acid sequence presented in Figure 2-(SEQ ID NO:2).

Appl. No. 10/027,000
Amdt. dated May 5, 2004
Reply to Office action of February 5, 2004

Page 6

9. [Currently Amended] A vector comprising ~~including~~ the expression construct of Claim 8.

10. [Original] A vector comprising an isolated polynucleotide of Claim 2, operably linked to control sequences recognized by a host cell transformed with the vector.

11. [Original] A host cell transformed with the vector of Claim 9.

12. [Original] A host cell transformed with the vector of Claim 10.

13. [Original] The host cell of Claim 12, which is a prokaryotic cell.

14. [Original] The host cell of Claim 12, which is a eukaryotic cell.

15. [Original] A recombinant host cell comprising a polynucleotide of Claim 2.

16. [Original] The recombinant host cell of Claim 15, which is a prokaryotic cell.

17. [Original] The recombinant host cell of Claim 15, which is a eukaryotic cell.

18. [Cancelled]

19. [Original] A method of producing an enzyme having β -glucosidase activity, comprising:

- (a) stably transforming a host cell with an expression vector comprising a polynucleotide as defined in Claim 2;
- (b) cultivating said transformed host cell under condition suitable for said host cell to produce said β -glucosidase; and
- (c) recovering said β -glucosidase.

20. [Original] The method of Claim 19 wherein the host cell is a filamentous fungi or yeast cell.

21. [Cancelled]

Appl. No. 10/027,000
Amdt. dated May 5, 2004
Reply to Office action of February 5, 2004

Page 7

22. [Cancelled]

23. [Currently Amended] An antisense oligonucleotide complementary to a messenger RNA that encodes a β -glucosidase 4 polypeptide having the sequence presented as SEQ ID NO:2, wherein upon exposure to a β -glucosidase-producing host cell, said oligonucleotide inhibits the production of β -glucosidase by said host cell compared to a control host cell.

24. [Original] The antisense oligonucleotide of Claim 23, wherein the host cell is a filamentous fungi.

25. [Cancelled]

26. [Previously Amended] A method of expressing a heterologous polypeptide having β -glucosidase activity in an *Aspergillus* species, comprising:

- (a) Providing a host *Aspergillus* with an expression vector comprising a polynucleotide encoding a signal sequence linked to a polynucleotide encoding a heterologous β -glucosidase according to claim 2, thereby encoding a chimeric polypeptide;
- (b) Cultivating said host *Aspergillus* under conditions suitable for said *Aspergillus* to produce said chimeric polypeptide, wherein said chimeric polypeptide is produced.

27 -36. [Cancelled]